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(54) Title: METHOD AND COMPOSITIONS FOR INHIBITING BIOSYNTHESIS OR BIOACTIVITY OF ENDOGENOUS STEROID SEX HORMONES IN HUMANS

(57) Abstract: Disclosed is a method of inhibiting biosynthesis or bioactivity of endogenous steroid sex hormones in both men and women involving the administration of a combination of phytosterol(s) and phytoestrogen(s) to inhibit enzymatic activity in the steroidogenic biosynthetic pathway that converts steroid progestins and androgens to more potent steroidal hormones, like estradiol and dihydrotestosterone. Also disclosed is a pharmaceutical composition useful for inhibiting biosynthesis or bioactivity of endogenous steroid sex hormones in humans. The pharmaceutical composition is formulated in a delivery system to deliver a dose of 50 to 250 mg of a phytosterol(s), such as campesterol, sitosterol, fucosterol, stigmasterol, stigmastanol, or stigmastadienone, or a derivative or conjugate of any of these, and 20 to 150 mg of a phytoestrogen(s), such as a lignan, isoflavone, flavone, or coumestan compound(s).

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METHOD AND COMPOSITIONS FOR INHIBITING BIOSYNTHESIS OR BIOACTIVITY OF ENDOGENOUS STEROID SEX HORMONES IN HUMANS

BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

1. THE FIELD OF THE INVENTION

This invention relates to the medical arts. In particular, the present invention relates to the field of hormone therapy and, more particularly, to the inhibition of steroidogenic biosynthesis and/or the bioactivity of steroid sex hormones.

2. DISCUSSION OF THE RELATED ART

Sex hormones include the female hormones (estrogens), in the form of female sex steroids, such as estradiol or estrone, and the male hormones (androgens), such as androstenedione and testosterone. These hormones are essential to the development of primary and secondary sexual characteristics. Estrogen provides women other physiological benefits. Women experiencing estrogen deficiency resulting from natural menopause, surgical removal of the ovaries, or ovarian failure resulting from chemotherapy, are known to be at higher than normal risk for atherosclerotic cardiovascular disease and osteoporosis. In addition, many postmenopausal women report reduced libido and sexual functioning. Estrogen replacement therapy (ERT) has long been used to prevent cardiovascular disease and osteoporosis in postmenopausal women and thus extend their life expectancy. Androgens, such as testosterone, are also produced by a woman's healthy ovary, albeit the serum levels of androgen in women are normally much lower than those found in men. In women, androgens also play a role in promoting cardiovascular health. (P.M. Sarrel, *Cardiovascular Aspects of Androgens in Women*, Sem. Reprod. Endocrinol. 16(2):121-27 [1998]), in preventing osteoporosis (Kaunitz, *The Role of Androgens in Menopausal Hormonal Replacement*, Menopause and Hormone Replacement Therapy 26(2):391-97 [1997]; Hughes *et al.*, *Combined Pharmaceutical Estrogen-Androgen-Progestin Oral Contraceptive*, U.S. Pat. No. 5,770,226), in maintaining sexual drive and function (J.K. Warnock *et al.*, *Female Hypoactive Sexual Desire Disorder Due to Androgen Deficiency*, Psychopharmacology Bulletin 33(4):761-66 [1997]), and in enhancing women's general energy level and sense of well-being. (M.J. Rosenberg *et al.*, *Estrogen-Androgen for Hormone Replacement*, J. Reprod. Med. 42(7):394-404 [1997]).

In men, androgens are essential for continued spermatogenesis, and in maintaining libido, positive mood, cognitive function, and skeletal and lean muscular mass and strength. Androgen replacement therapy is prescribed for hypogonadal men, who typically receive testosterone or a

derivative. (C. Wang and R.S. Swerdloff, *Androgen Replacement Therapy*, Ann. Med. 29(4):365-70 [1997]). Androgen replacement therapy also leads to increased body weight and improved quality of life for men and women suffering from AIDS wasting. (K. Miller *et al.*, *Transdermal Testosterone Administration in Women with Acquired Immunodeficiency Syndrome Wasting: A Pilot Study*, J. Clin. Endocrinol. Metab. 85(8):2717-25 [1998]; A.S. Dobs *et al.*, *Endocrine disorders in men infected with human immunodeficiency virus*, Am. J. Med. 84:61-15 [1988]).

However, steroid sex hormones are associated with increased disease risks. In women, estrogen, produced endogenously or provided by ERT, is associated with an increased risk for cancers of the breast and endometrium, endometriosis, thromboembolic disease, gall bladder disease, and, in some cases, idiosyncratic increases in blood pressure, hypercalcemia, hypercoagulability, and hypertriglyceridemia.

Testosterone and androstenedione are also associated with increased breast cancer risk in women. (A. Zeleniuch-Jacotte *et al.*, *Relation of Serum Levels of Testosterone and Dehydroepiandrosterone Sulfate to Risk of Breast Cancer in Postmenopausal Women*, Am. J. Epidemiol. 145(11):1030-38 [1997]; F. Berrino *et al.*, *Serum Sex Hormone Levels After Menopause and Subsequent Breast Cancer*, J. Natl. Cancer Inst. 88(5):291-96 [1996]; J.F. Dorgan *et al.*, *Relation of Prediagnostic Serum Estrogen and Androgen Levels to Breast Cancer Risk*, Cancer Epidemiol., Biomarkers Prevent. 5(7):533-39 [1996]; D. Wysowski *et al.*, *Sex Hormone Levels in Serum in Relation to the Development of Breast Cancer*, Am. J. Epidemiol. 126(5):791-99 [1987]).

And in men, testosterone and androstenedione are associated with cancers of the prostate and male breast and with benign prostatic hypertrophy (BPH) resulting in urinary obstruction. These increased disease risks derive from the fact that aromatizable androgens, such as testosterone, can be converted to steroidal estrogens by aromatase activity. (D.F. Dimick *et al.*, *A comparative study of the metabolic fate of testosterone, 17a-methyl-testosterone, 19-nor-testosterone, 17a-methyl-19-nor-testosterone and 17a-methyl--estr-5(10)-ene-17-b-OL-3-one ion normal males*, Clin. Chim. Acta 6:63-67 [1961]).

The aromatase cytochrome P450 complex (E.C. 1.14.13; $P450_{AROM}$; the product of the CYP19 gene, also known as estrogen synthetase) is a microsomal enzyme that catalyzes the synthesis of estrogens from aromatizable androgens. Specifically, aromatase catalyses three consecutive hydroxylation reactions that convert C-19 androgens to aromatic C-18 estrogenic steroids, for example, androstenedione to estrone and testosterone to estradiol. (Reviewed in S. Chen, *Aromatase and Breast Cancer*, Frontiers in Bioscience 3:d922-d933 [1998]). Aromatase is expressed in a tissue-specific manner. In premenopausal women aromatase is mainly expressed in the ovaries, but significant expression also occurs in breast tissue. In postmenopausal women and in men, aromatase activity is much lower and concentrated in adipose tissue and skin cells. Aromatase is also expressed in the prostates of men with prostate cancer and BPH, as well as 5α-reductase, the enzyme that catalyzes the conversion of testosterone to the androgenically more potent dihydrotestosterone. (M.

Tsugaya *et al.*, *Aromatase mRNA levels in benign prostatic hyperplasia and prostate cancer*, Int. J. Urol. 3(4):292-96 [1996]; P. Negri-Cesi *et al.*, *Presence of 5-alpha-reductase isozymes and aromatse in human prostate cancer cells and in benign prostate hyperplastic tissue*, Prostate 34(4):283-91 [1998]; M. Hiramatsu *et al.*, *Aromatase in hyperplasia and carcinoma of the human prostate*, Prostate 31(2):118-24 [1997]).

The use of inhibitors of aromatase and 5α-reductase has been taught in a combination therapy for preventing or treating BPH. (F. Labrie, Combination therapy for the prophylaxis and/or treatment of benign prostatic hyperplasia, U.S. Patent Nos. 5,817,649 and 5,595,985; K. Suzuki *et al.*, *Effect of dual inhibition of 5-alpha-reductase and aromatase on spontaneously developed canine prostatic hypertrophy*, Prostate 37(2):70-76 [1998]). But treatment of established BPH with an aromatase inhibitor (atemestane) alone was reported to be clinically ineffective. (A. Radlmaier *et al.*, *Estrogen reduction by aromatase inhibition for benign prostatic hyperplasia: results of a double-blind, placebo-controlled, randomized clinical trial using two doses of the aromatase inhibitor atemestane. Atemestane Study Group*, Prostate 29(4):199-208 [1996]).

Aromatase is expressed at higher levels in human breast cancer tissue than in normal breast tissue, and estrogens produced via aromatization in situ in breast tissue play a more important role in the development of breast cancer than circulating estrogen. In breast cancer cells, aromatase activity resulting in estrogen biosynthesis stimulates tumor growth in both an autocrine and a paracrine manner. (X.Z. Sun *et al.*, *Autocrine and paracrine actions of breast tumor aromatase. A three-dimensional cell culture study involving aromatase transfected MCF-7 and T-47D cells*, J. Steroid Biochem. Mol. Biol. 63(1-3):29-36 [1997]; W.E. Burak *et al.*, *Androgens influence estrogen-induced responses in human breast carcinoma cells through cytochrome P450 aromatase*, Breast Cancer Res. Treat. 44(1):57-64 [1997]).

In view of this fact, aromatase inhibiting drugs have been used for prevention and treatment of breast cancer in cases where anti-estrogen therapy has been ineffective. These aromatase inhibitors include imidazole derivatives, Letrozole, Vorozole, Anastrozole, substituted androstenedione derivatives, such as 4-hydroxyandrostenedione (LENTERON), and aminoglutethimide. (S. Chen [1998]; K.S. Hirsch *et al.*, *Method of inhibiting aromatase*, U.S. Patent No. 4,766,140; F. Labrie *et al.*, *Aromatase inhibitors*, U.S. Patent No. 5,227,375; K. Niimura *et al.*, *Method of inhibiting aromatase*, U.S. Patent No. 5,547,973; A.J. Karjalainen *et al.*, *Selective aromatase inhibiting compounds*, U.S. Patent No. 5,703,109; F. Faustini *et al.*, *Steroidic aromatase inhibitors*, U.S. Patent No. 4,771,043). Aminoglutethimide is a non-specific aromatase inhibitor that also inhibits enzymatic activity by cytochrome P450 cholesterol side chain cleavage complex ($P450_{SCC}$; desmolase). (S. Chen [1998]). This enzyme, located on the inner mitochondrial membrane, catalyzes the first step of steroidogenesis leading to the biosynthesis of the steroid sex hormones, i.e., the conversion of cholesterol to pregnenolone and isocapraldehyde by sequential oxidations of the cholesterol side chain.

Some disadvantages have emerged with respect to current aromatase inhibitor drug therapies

for women. Only about twenty to thirty percent of women who fail to respond to anti-estrogen therapy respond to aromatase inhibitors. In addition, premenopausal women taking aromatase inhibitors, frequently experience a feedback increase in luteinizing hormone and follicle-stimulating hormone, which may counteract the effect of some aromatase inhibitors. In some patients, aminoglutethimide can actually increase aromatase activity in breast tumors. W.R. Miller and J. O'Neill, *The importance of local synthesis of estrogen within the breast*, Steroids 50:537-48 [1987]).

Many plants and some fungi naturally produce compounds similar in structure to estrogens that produce a physiological estradiol-like effect in mammals. These so-called phytoestrogen compounds affect mammalian physiology by several mechanisms, including estrogen receptor agonism and possible antagonism, by their receptor-independent antioxidant properties, and by inhibition of several enzymes, including a number of enzymes involved in cell-signaling and proliferation, such as tyrosine kinase and DNA topoisomerases I and/or II, as well as aromatase. (S. Chen [1998]; Y.C. Kao *et al.*, *Molecular basis of the inhibition of human aromatase [estrogen synthetase] by flavone and isoflavone phytoestrogens: a site-directed mutagenesis study*, Environ. Health Perspect. 106(2):85-92 [1998]; D.R. Campbell and M.S. Kurzer, *Flavonoid inhibition of aromatase enzyme activity in human preadipocytes*, J. Steroid. Biochem. Mol. Biol. 46(3):381-88 [1993]; C. Pelissero *et al.*, *Effects of flavonoids on aromatase activity, an in vitro study*, J. Steroid Biochem. Mol. Biol. 57(3-4):215-23 [1996];G.G. Kuiper *et al.*, *Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta*, Endocrinol. 139(10):4252-63 [1998]; R.J. Miksecek, *Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor*, J. Steroid Biochem. Mol. Biol. 49(2-3):153-60 [1994]; S. Stahl *et al.*, *Phytoestrogens act as estrogen agonists in an estrogen-responsive pituitary cell line*, Toxicol. Appl. Pharmacol. 152(1):41-48 [1998]; M.E. Baker *et al.*, *Flavonoids inhibit estrogen binding to rat alpha-fetoprotein*, Proc. Soc. Exp. Biol. Med. 217(3):317-21 [1998]); G. Peterson and S. Barnes, *Genistein inhibits both estrogen and growth factor-stimulated proliferation of human breast cancer cells*, Cell Growth and Differ. 7(10):1345-51 [1996]; C. Wang, *et al.*, *Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes*, J. Steroid Biochem. Mol. Biol. 50(3-4):205-12 [1994]).

Phytoestrogens include lignan, isoflavone, flavone, and coumestan compounds, and their metabolites, such as equol. The lignans, isoflavones, flavones, and coumestans have structures that are conformationally similar to the structure of 17-β-estradiol, thus they act as estrogen analogues with respect to estrogen receptor binding sites. (C.L. Hughes *et al.*, *Dietary soy phytoestrogens and the health of menopausal women: overview and evidence of cardioprotection from studies in no-human primates*, In: *Progress in the Management of the Menopause*, B.G. Wren [ed.], The Parthenon Publishing Group, pp. 30-39 [1997]). Some dietary phytoestrogens were also reported to reduce serum cholesterol and hypercholesterolemic atherosclerosis in mammals. (K. Prasad, *Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed*, Circulation 99(10):1355-62 [1999]).

The lignans have core structural elements related to both the coumestans and the isoflavones,

but their affinity for estrogen receptor binding sites is less than either of these or the flavones. Naturally occurring lignans are obtained from numerous sources including, oilseed plants, such as sesame or linseed, seaweeds, flax seed flour or defatted flax seed meal, whole legumes, legume hulls, cereal brans, whole grain cereals, vegetables, fruits, including nuts and berries. (L.U. Thompson *et al.*, *Mammalian lignan production from various foods*, Nutr. Cancer 16(1):43-52 [1991]). Sources rich in coumestans include grapes and currants, wine, or apples. (T.L Wadworth and D.R. Koop, *Effects of wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages*, Biochem. Pharmacol. 57(8):941-49 [1999]).

Legumes and grains are rich in dietary phytoestrogens, with soy content of the isoflavones genistein, daidzein, and their conjugates on the order of 3 mg/gram of soy protein. (C.L. Hughes *et al.* [1997]). Fermentation of isoflavone containing foods, such as fermentation of soy to tempeh, decreases the total isoflavone content but increases the bioavailability of isoflavones. (A.M. Hutchins *et al.*, *Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products*, J. Am. Diet. Assoc. 95(5):545-51 [1995]). Dietary phytoestrogens are readily absorbed by humans and circulate in blood plasma at levels ranging up to hundreds of nmol/L in persons consuming high soy diets. Many peoples of the Pacific Rim traditionally eat a high soy diet, and the cancer-preventative properties of phytoestrogens in soy, such as genistein, are thought to be associated with the fact that these populations suffer a lower incidence of breast cancer, prostate cancer, colon cancer, and arteriosclerotic cardiovascular disease than Western populations. (A.M. Hutchins *et al.* [1995]; J.M. Cline and C.L. Hughes, *Photochemical for the prevention of breast and endometrial cancer*, Cancer Treat. Res. 94:107-34 [1998]); D. Ingram *et al.*, *Case-control study of phyto-oestrogens and breast cancer*, Lancet 350:990-94 [1997]; G. Peterson and S. Barnes [1996]; M.J. Messina *et al.*, *Soy intake and cancer risk: a review of the in vitro and in vivo data*, Nutr. Cancer 21(2):113-31 [1994]; J. Nutr. 128(10):1589-92 [1998]; A.H. Lichtenstein, *Soy protein, isoflavones and cardiovascular disease risk*, J. Nutr. 128(10):1589-92 [1998]; H. Adlercreutz *et al.*, *Dietary phyto-oestrogens and the menopause in Japan*, Lancet 339:1233 [1997]; Z.M. Shao *et al.*, *genistein exerts multiple suppressive effects on human breast carcinoma cells*, Cancer Res. 58(21):4851-57 [1998]; M.C. Pagliacci *et al.*, *Growth-inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells*, Eur. J. Cancer 30A(11):1675-82 [1994]).

Recognizing the potential health benefits of phytoestrogens, Kelly *et al.* taught a method for treating or reducing the predisposition to benign breast disease, prostate cancer, or elevated serum cholesterol, by administering a composition comprising at least two phytoestrogens or phytoestrogen-derived isoflavones. (Kelly *et al.*, *Health supplements containing phyto-oestrogens, analogues or metabolites thereof*, U.S. Patent 5,830,887). Hughes *et al.* described a pharmaceutical preparation for oral delivery of a combination of mammalian estrogen and soy-derived phytoestrogen to reduce the risk of coronary heart disease and osteoporosis in women. (Hughes *et al.*, *Dietary phytoestrogen in estrogen replacement therapy*, U.S. Patent No. 5,516,528). Jackson *et al.* described dietary supplements for women containing phytoestrogens. (Jackson et al., *Dietary supplements*, U.S.

Patents 5,654,011; Jackson et al., Method of dietary supplementation, U.S. Patent No. 5,807,586). Shlyankevich taught compositions that contained phytoestrogen compounds for controlling the stimulation of estrogen production. (Shlyankevich, *Pharmaceutical compositions for the management of premenstrual syndrome and alleviation of menopausal disorders*, U.S. Patent No. 5,569,459). In addition, Gorbach taught the use of isoflavonoid compounds for the treatment and prevention of Alzheimer dementia and reduced cognitive function in the aged. (Gorbach, *Isoflavonoids for treatment and prevention of Alzheimer dementia and reduced cognitive functions*, U.S. Patent No. 5,733,926).

Plant sterols (phytosterols) are another common dietary component that may have hormonal effects in humans. A few plants contain modest levels of estrogenic (C_{18}), androgenic (C_{19}), or progestagenic (C_{21}) steroids, but sterols with longer side chains (C_{26}-C_{30}) are quite common, many having a structure similar to that of cholesterol, including campesterol, β-sitosterol, fucosterol, stigmasterol, stigmastanol, and stigmastadienone. (Reviewed in C.L. Hughes, *Plant Sterols: are they mammalian reproductive hormones?*, Sex Steroids 3(1):285-91 [1992]). Dietary phytosterols are absorbed, transported and metabolized like cholesterol; they suppress circulating levels of free cholesterol in humans and other mammals, and thus may have a general positive effect on cardiovascular health. (C.B. Kallen *et al.*, *Steroidogenic acute regulatory protein (StAR) is a sterol transfer protein*, J. Biol. Chem. 273:26285-88 [1998]; P.J. Jones and F. Ntanios, *Comparable efficacy of hydrogenated versus non-hydrogenated plant sterol esters on circulating cholesterol levels in humans*, Nutr. Rev. 56(8):245-48 [1998]).

See taught a sitosterol-apple pectin complex for inhibiting fat and cholesterol absorption from the gut. (J.R. See, *Dietary supplement incorporating β-sitosterol and pectin*, U.S. Patent No. 5,747,464).

Thakkar *et al.* taught a pharmaceutical preparation comprising a dispersible powder for oral administration consisting essentially of sitosterol (a form of phytosterol) and an excipient. (Thakkar *et al.*, *Pharmaceutical dispersible powder of sitosterols and a method for the preparation thereof*, U.S. Patent No. 3,881,005).

Jandacek taught plant sterols with enhanced cholesterol-lowering properties in disclosing a food composition comprising at least one edible oil in admixture with a plant sterol. (Jandacek, *Edible oils having hypocholesterolemic properties*, U.S. Patent No. 5,865,939)

Weigand taught a method for reducing serum cholesterol and other lipids by administering a combination of sitosterol and a steroid compound. (Weigand, *Reducing cholesterol levels with sitosterols and cholanic acid*, U.S. Patent No. 3,852,440).

There is evidence that phytosterols are effective in treating BPH, (R.R. Berges *et al.*, *Randomised, placebo-controlled, double-blind clinical trial of beta-sitosterol in patients with benign prostatic hyperplasia*, Lancet 345(8964):1529-32 [1995]; F.C. Lowe and J.C. Ku, *Phytotherapy in treatment of benign prostatic hyperplasia*, Urol. 48(1):12-20 [1996]; K.F. Klippel *et al.*, *A multi-centric, placebo-controlled, double-blind clinical trial of beta-sitosterol (phyosterol) for the treatment*

of benign prostatic hyperplasia. German BPH-Phyto Study group, Br. J. Urol. 80(3):427-32 [1997]).

β-Sitosterol and other phytosterols are thought to act either as substrates or inhibitors of $P450_{SCC}$ and thus may affect the kinetics of cholesterol biosynthesis in a yet unknown manner; they are, therefore, thought to be likely modulators of steroidal hormone production. (C.L. Hughes [1992]). However, the effect of phytosterols has been thought to be primarily estrogenic. The sterol content of vegetable oils ranges from 0.5% to 1%, and typical human consumption in Western countries is 150-250 mg of phytosterols per day, consequently, dietary phytosterols may be converted to a substantial amount of estradiol by endogenous steroidogenic metabolism or by intestinal bacteria before absorption into the blood stream, posing an *increased* risk of breast and endometrial cancers. Id. β-Sitosterol was reported to bind to cytosolic estrogen receptor binding sites in rat liver and uterine cells in vitro and to have weak estrogenic effects at low dose upon rats in vivo. (E.R. Rosenblum *et al., Assessment of the estrogenic activity of phytoestrogens isolated from bourbon and beer*, Alcoholism Clin. Exp. Res. 17(6):1207-09 [1993]). However, it was reported that in goldfish treated with β-sitosterol, plasma androgen levels, in males, and 17-β-estradiol levels, in females, were significantly decreased, gonadotropin levels were increased in males, and basal and hCG-stimulated pregnenolone and testosterone levels were reduced in testes pieces removed from β-sitosterol-treated fish. (D.L. MacLatchy and G.J. Van Der Kraak, *The phytoestrogen beta-sitosterol alters the reproductive endocrine status of goldfish*, Toxicol. Appl. Pharmacol. 134(2):305-12 [1995]). These results implied that β-sitosterol reduced the biosynthesis of steroid sex hormones through effects on cholesterol availability or the activity of $P450_{SCC}$.

There is a need for a method of down-regulating the biosynthesis of steroid sex hormones and or reducing their bioactivity in humans, thus, lowering the risk of cancers of the breast, endometrium, and prostate, and of BPH, while simultaneously preserving the benefits to cardiovascular and skeletal health, libido and energy, and cognitive function that are associated with aromatizable androgens and estrogens. This and other features and benefits provided by the present invention will now be described.

SUMMARY OF THE INVENTION

The present invention is directed to a method of inhibiting biosynthesis or bioactivity of endogenous steroid sex hormones in both men and women. The method involves a combined administration of phytosterol(s) and phytoestrogen(s). The invention is particularly beneficial in the prevention of breast and endometrial cancers in women, for which the estrogenic steroid, estradiol, is a known risk factor. The method is applicable to adult premenopausal and postmenopausal women, including postmenopausal women receiving estrogen replacement therapy. Using the method, these women can continue to enjoy the benefits of improved cardiovascular and skeletal health, provided by ERT, with lower breast and endometrial cancer risks. The method is also beneficial to virtually all men over 50 years old in preventing or reducing benign prostatic hyperplasia (BPH) and prostatic cancer. The method provides cardiovascular benefits to both men and women

from the activity of phytosterols and phytoestrogens in lowering serum cholesterol and in ameliorating dyslipidemia, shifting lipid profiles toward relatively higher HDL and lower LDL values.

The present invention is also related to pharmaceutical compositions useful for inhibiting the biosynthesis or bioactivity of endogenous steroid sex hormones in humans. The pharmaceutical composition is formulated in a delivery system to deliver a dose of 50 to 250 mg of phytosterol compound(s) and 20 to 150 mg of phytoestrogen compound(s), at which beneficial levels there is essentially no toxic risk.

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention is directed to the combined use of phytosterol and phytoestrogen compounds in a method of inhibiting biosynthesis or bioactivity of an endogenous steroid sex hormone in a human subject. The method involves administering to a human subject a combination of a dose of at least one phytosterol compound together with a dose of at least one phytoestrogen compound in an amount sufficient to inhibit the biosynthesis or bioactivity of an endogenous steroid sex hormone, such as an estrogen, for example, estradiol or estrone, or an aromatizable or non-aromatizable androgen, such as, androstenedione, dehydroepiandrosterone, testosterone, or dihydrotestosterone. But also included among the endogenous steroid sex hormones to which the method is directed are the progestins, for example, progesterone and 17-hydroxyprogesterone, that are precursors to estrogen and androgen biosynthesis.

The phytosterol(s) and phytoestrogen compound(s) are administered substantially simultaneously, such that effective amounts of phytosterol and phytoestrogen compounds, or their derivatives, are present together in the human subject's serum for at least a period of 1 to 8 hours daily, and most preferably 24 hours daily.

Preferably, but not necessarily, the method involves administering to a human subject a pharmaceutically acceptable composition of the present invention, containing a combination of at least one phytosterol compound and at least one phytoestrogen compound.

Useful phytosterol and phytoestrogen compounds are primarily derived from natural or transgenic plant sources. But some can also be derived from fungal sources, e.g., the isoflavones zearalenone and β-zearalenol, metabolites derived from the fungus *Fusarium*.

Other useful steroidogenic biosynthesis-inhibiting or steroid bioactivity-inhibiting phytosterol or phytoestrogen compounds are bacterial metabolites of plant- or fungus-derived phytosterol or phytoestrogen compounds. For example, bacterial action in the mammalian gastrointestinal tract leads to conversion of various lignan species to enterodiol and enterolactone, which are the major lignan species found in human serum and excreted in human and other mammalian urine after ingestion of a variety of dietary lignans, e.g., matairesinol or secoisolariciresinol diglycoside. (S.P. Borriello *et al.*, *Production and metabolism of lignans by human faecal flora*, J. Appl. Bacteriol. 58(1):37-43 [1985]; S.E. Rickard *et al.*, *Dose-dependent production of mammalian lignans in rats*

and in vitro from the purified precursor secoisolariciresinol diglycoside in flaxseed, J. Nutr. 126(8):2012-19 [1996]; M. Nose *et al., Structural transformation of lignan compounds in rat gastrointestinal tract*, Planta Med. 58(6):520-23 [1992]).

Other useful phytoestrogen species obtained as bacterial metabolites include aglycones of flavone and coumestan glucoside precursors. (E.g., D.H. Kim *et al., Intestinal bacterial metabolism of flavonoids and its relation to some biological activities*, Arch. Pharm. Res. 21(1):17-23 [1998]; H. Schneider *et al., Anaerobic transformation of quercetin-3-glycoside by bacteria from the human intestinal tract*, Arch. Microbiol. 171(2):81-91 [1999]; V.D. Bokkenheuser *et al., Hydrolysis of dietary flavonoid glycosides by strains of intestinal Bacteroides from humans*, Biochem. J. 248(3):953-56 [1987]; A.A. Aziz *et al., Absorption and excretion of conjugated flavonols, including quercetin-4'-O-beta-glucoside and isorhamnetin-4-'-O-beta glucoside by human volunteers after consumption of onions*, Free Radic. Res. 29(3):257-69 [1998]).

However, whether or not a particular species of phytosterol compound or phytoestrogen compound is metabolized in any way or deconjugated in a human gastrointestinal tract before its entry into the blood stream does not limit the embodiments of phytosterol or phytoestrogen compounds that can be used in accordance with the present invention. The present invention is not committed to any particular mechanism by which a particular compound operates to inhibit biosynthesis (steroidogenesis) or bioactivity of one or more steroid sex hormones.

The phytosterol compound(s) and phytoestrogen compound(s) are obtained commercially. Alternatively, they are purified from naturally occurring sources by known biochemical means. Synthetic or semi-synthetic versions or derivatives of phytosterol or phytoestrogen compounds are also useful in the present pharmaceutical composition and methods of synthesis or derivatization are known. (E.g., J.L. Belletire *et al., The role of anion coupling in the synthesis of dibenzylbutane lignans*, J. Nat. Prod. 55(2):184-93 [1992]).

Useful phytosterol compounds include campesterol, sitosterol, fucosterol, stigmasterol, stigmastanol, or stigmastadienone. A most preferred phytosterol compound is sitosterol. β-sitosterol is preferred, but α- or γ-sitosterol are also useful forms of sitosterol. Other preferred phytosterol compounds include phytosterol derivatives or conjugates, for example, the conjugate β-sitosterol-β-D-glucopyranoside. In the present pharmaceutical composition a mixture of phytosterol compounds can also be employed.

Useful phytoestrogen compounds include lignan compounds, isoflavone compounds, flavone compounds, or coumestan compounds, or derivatives, conjugates of any of these. Phytoestrogens include free (unconjugated) or conjugated forms, for example, sulfated, or sulfonated phytoestrogen conjugates, or glucoside, glucuronide, or sulfoglucuronide phytoestrogen conjugates. A mixture of phytoestrogen compounds can also be employed.

Suitable lignan compounds include sesamin, justiciresinol, lariciresinol, isolariciresinol, secoisolariciresinol, O-demethylsecoisolariciresinol, didemethylsecoisolariciresinol, demethoxysecoisolariciresinol, matairesinol, syringaresinol, episyringaresinol, diasyringaresinol,

massoniresinol, lirioresinol, entrodiol, enterolactone, gomisin A, gomisin C, gomisin D, nordihydroguaiaretic acid, 3'-O-methyl nordihydroguaiaretic acid, arctigenin, or 3'-O-demethylarctigenin, or derivatives or conjugates of any of these. Examples of useful lignan conjugates include syringaresinol- β-D-glucoside, massoniresinol 4"-O-β-D-glucopyranoside, secoisolariciresinol diglycoside, and ramontoside, a butyrolactone lignan disaccharide. (E.g., see also G. Brunner *et al.*, *Enzymatic synthesis and chromatographic purification of lignan glucuronides*, Biomed. Chromatogr. 1(2):89-92 [1986]).

A most preferred phytoestrogen is an isoflavone compound. Preferred are genistein, daidzein, biochanin A, glycitein, zearalenone, beta-zearalenol, formononetin, O-desmethylangolensin, ipriflavone, apigenin, phloretin, baicalein, alpinumisoflavone, hydroxyalpinumisoflavone, or a derivative or conjugate of any of these. An example of a useful phytoestrogen derivative is equol, which is a metabolite derived primarily from daidzein. Other useful derivatives include dihydrodaidzein, tetrahydrodaidzein, dihydrogenistein, 2-dehydro-O-demethylangolensin.

In another embodiment, the phytoestrogen is a flavone compound. Useful examples include kaempferol, galangin, fisetin, morin, chrysin, tectochrysin (5-hydroxy-7-methoxyflavone), isoprunetin, wighteone, laburnetin, diosmetin, genistin, genisteone, ephedroidin, baicalin, puerarin, poncirin, hesperidin, naringin, isorhamnetin, norwogenin, 2,5-dihydroxy-6,7-dimethoxyflavonone, and their derivatives and conjugates. (E.g., L. Pistelli *et al.*, *Flavonoids from Genista ephedroides*, J. Nat. Prod. 61(11):1404-06 [1998]). Also preferred are xanthohumol, isoxanthohumol, desmethoxyxanthohumol, naringenin, 6- and 8-prenylnaringenins, 6-geranylnaringenin, which are derived from hops and beer. (J.F. Stevens *et al.*, *Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry*, J. Chromatogr. A 832(1-2):97-107 [1999]).

Useful flavone conjugates include flavone glucosides, for example, 7-methoxy-flavone-5-O-glucosides. (E.g., A. Zahir *et al.*, *Five new flavone 5-O-glycosides from Lethedon tannaensis: lethesides and lethediosides*, J. Nat. Prod. 62(2):241-43 [1999]). Other useful embodiments of a flavone conjugate include kaempferol-3-O-beta-D-glucopyranoside, isorhamnetin-4'-O-β-glucoside, and flavonone sulfonates, for example, galangin-8-sulfonate, galangin-3-O-beta-D-glucoside-8-sulfonate, and kaempferol-8-sulfonate.

In another embodiment, the phytoestrogen compound is a coumestan compound(s). Examples include coumestrol, quercetin, resveratrol, rutin, myricetin, luteolin, or derivatives or conjugates of any of these. Examples of useful coumestan derivatives include 3-O-methylquercetin and 3',4'-di-O-benzyl-3-O-methylquercetin. And useful coumestan conjugates include, for example, quercetin-3-O-β-D-glucopyranoside, quercetin-4'-O-β-D-glucopyranoside, quercetin-3,4-diO-β-D-glucoside, quercetin-7-O-β-D-glucopyranoside, 4-(G)-α-glucopyranosylrutin (G-rutin).

Progress in pharmacogenetics has shown that human genetic variation underlies different individual responses to treatment within a population. (Reviewed in G. Alvan, *Genetic polymorphisms in drug metabolism*, J. Int. Med. 231:571-73 [1992]; P.W. Kleyn and E.S. Vesell,

Genetic variation as a guide to drug development, Science 281:1820-22 [1998]). Whether a particular human subject responds to a particular phytosterol/phytoestrogen combination is determined during a trial interval of 2 to 8 weeks duration.

Any suitable method is used to determine that biosynthesis or bioactivity of endogenous steroid sex hormones has been inhibited. For example, "direct" measurements can be made of serum or other tissue-specific levels of steroid sex hormones, such as estradiol, estrone, estrone sulfate; testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulfate. Alternatively, "indirect" measurements can be made of hormone dependent markers in the blood plasma such as sex hormone binding globulin (bound or unbound) or prostate-specific antigen. Alternatively, "target tissue" or "disease" measurements can be made of disease responses, for example, ultrasonic measurements showing the shrinkage of uterine leiomyomata (fibroids) or diminished prostate size as determined by ultrasonic or digital exam, in response to treatment in accordance with the present method. Alternatively, biopsy samples can be assayed for expression or activity of enzymes of the steroidogenic biosynthetic pathway, such as aromatase, $P450_{SCC}$, 17-hydroxysteroid dehydrogenase, 17α-hydroxylase/C_{17-20} lyase, or 5α-reductase, in specific tissues such as breast, uterus, prostate, or any other tissue of interest. These examples are merely illustrative, and not an exhaustive list, of the methods known to the skilled artisan of determining that biosynthesis or bioactivity of endogenous steroid sex hormones has been inhibited in a particular human subject. If there has been no detectable reduction in the levels of steroid sex hormones, for example estradiol, or no change in the relative ratios among sex steroids has occurred within two to eight weeks, the practitioner should try a different combination of phytosterol and phytoestrogen compound(s).

An effective dose for inhibiting biosynthesis or bioactivity of an endogenous steroid sex hormone in a human subject is an amount sufficient to inhibit the level of one or more steroid sex hormone(s) in serum or in a localized tissue, or to inhibit bioactivity (e.g., by specific receptor-mediated or non-receptor-mediated mechanisms of bioactivity) of a steroid sex hormone, compared to the level or bioactivity in the subject before combined administration to the subject of phytosterol and phytoestrogen compound(s) in accordance with the present method. Alternatively, a dose effective to inhibit biosynthesis (steroidogenesis) in a human subject is an amount sufficient to reduce expression or activity of aromatase, $P450_{SCC}$, 17-hydroxysteroid dehydrogenase, 17α-hydroxylase/C_{17-20} lyase, 5α- reductase, or other enzyme of the steroidogenic pathway, in a tissue of the subject, for example, breast or prostate tissue. The effective dose for each human subject will depend upon the size and physiologic reactions of the subject to whom or to which the pharmaceutical preparations of the present invention are administered, and these reactions to the administered dose can be monitored by the prescribing physician. Typically, a phytosterol compound is delivered in an effective dose of 1 to 5 milligrams per kilogram of body weight per day, and the phytoestrogen compound is delivered in a dose of 0.5 to 2 milligrams per kilogram of body weight per day.

The phytosterol and phytoestrogen compounds are administered by any suitable method. Representative methods include giving, providing, feeding or force-feeding, dispensing, inserting, prescribing, furnishing, treating with, taking, swallowing, eating or applying a pharmaceutical composition of the present invention.

A preferred embodiment of the present method involves a systemic delivery route, i.e., a route whereby delivery of a phytosterol compound and a phytoestrogen compound are delivered to body tissues primarily via the blood stream. Entry of phytosterol and phytoestrogen compounds into the blood stream of a human can occur by any route, system, device, method or mechanism. For the purposes of the present invention, a systemic delivery route can also include delivery through the skin, mucosa or epithelium of the mouth including the sublingual epithelium, the rectum, or the vaginal epithelium.

Systemic delivery systems include, but are not limited to, ingestion, injection, or intravenous drip, most conventionally. A preferred ingestive delivery system is a pharmaceutically acceptable food additive or food supplement for humans, formulated as a powder, tablet, capsule or caplet.

Other useful systemic delivery systems are known and include, but are not limited to, implant; adhesive transdermal patches; topical creams, gels or ointments for transdermal delivery; transmucosal delivery matrices or suppositories or gels. It is contemplated that the compositions of the present invention are formulated to deliver an effective dose of a phytosterol compound and a phytoestrogen compound by these or any other pharmaceutically acceptable systemic delivery system.

The present invention also relates to a pharmaceutical composition comprising a combination of at least one phytosterol compound and at least one phytoestrogen compound. As well as the phytosterol compound(s) and phytoestrogen compound(s), the pharmaceutical composition of the present invention can optionally contain pharmaceutically acceptable solvent(s), adjuvant(s) or non-medicinal carrier(s), binder(s), thickener(s), or filler substance(s) that are known to the skilled artisan. Common fillers include, but are not limited to, sucrose or lactose, or polymeric substances like starch. Also contemplated are additional medicinal or nutritive additives in combination with a phytosterol and phytoestrogen compound, as may be desired to suit the more particular needs of the practitioner.

The present pharmaceutical composition can be formulated and manufactured at more than one concentration, such that modular incremental amounts of phytosterol compound(s) and phytoestrogen compound(s) are easily administered. Preferably, the composition is formulated in a delivery system to deliver a dose of 50 to 250 mg of phytosterol compound(s) and 20 to 150 mg of phytoestrogen compound(s). Most preferably, the composition is formulated in a delivery system to deliver a dose of 175 to 225 milligrams of the phytosterol compound(s) and 75 to 125 milligrams of the phytoestrogen compound(s). These preferred dose ranges provide beneficial effect with essentially no toxic risk.

A preferred embodiment of the present pharmaceutical composition is formulated for a systemic delivery system such as, but not limited to, ingestive, injection, or intravenous drip systems. A preferred ingestive delivery system is a pharmaceutically acceptable food additive or food supplement for humans, formulated as a powder, tablet, capsule or caplet.

Another preferred embodiment of the present pharmaceutical composition is a topical cream, ointment or gel to be applied to the skin. In this embodiment, a composition of the present invention comprises a phytosterol compound and a phytoestrogen compound in a pharmaceutically acceptable delivery system comprising a permeation or penetration enhancer, such as polyethylene glycol monolaurate, dimethyl sulfoxide, N-vinyl-2-pyrrolidone, N-(2-hydroxyethyl)-pyrrolidone, or 3-hydroxy-N-methyl-2-pyrrolidone. A variety of conventional thickeners often used in creams, ointments and gels, such as, but not limited to, alginate, xanthan gum, or petrolatum, may also be employed in this embodiment of a composition of the present invention.

Another preferred embodiment of the composition of the present invention is a formulation for systemic transmucosal delivery of a phytosterol compound and a phytoestrogen compound. A variety of pharmaceutically acceptable systems for transmucosal delivery of therapeutic agents are known in the art and are compatible with the practice of the present invention. (Heiber *et al.*, *Transmucosal delivery of macromolecular drugs*, U.S. Pat. Nos. 5,346,701 and 5,516,523; Longenecker *et al.*, *Transmembrane formulations for drug administration*, U.S. Pat. No. 4,994,439). Transmucosal delivery devices may be in free form, such as a cream, gel, or ointment, or may comprise a determinate form such as a tablet, patch, or troche. For example, delivery of a phytosterol compound and a phytoestrogen compound may be via a transmucosal delivery system comprising a laminated composite of, for example, an adhesive layer, a backing layer, a permeable membrane defining a reservoir containing a phytosterol compound and a phytoestrogen compound, a peel seal disc underlying the membrane, one or more heat seals, and a removable release liner. (Ebert *et al.*, *Transdermal delivery system with adhesive overlay and peel seal disc*, U.S. Pat No. 5,662,925; Chang *et al.*, *Device for administering an active agent to the skin or mucosa*, U.S. Pat. Nos. 4,849,224 and 4,983,395).

Alternatively, a tablet or patch for delivery through the oral mucosa can comprise an inner layer containing the therapeutic agent of choice, a permeation enhancer, such as a bile salt or fusidate, and a hydrophilic polymer, such as hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxyethylcellulose, dextran, pectin, polyvinyl pyrrolidone, starch, gelatin, or any of a number of other polymers known to be useful for this purpose. This inner layer can have one surface adapted to contact and adhere to the moist mucosal tissue of the oral cavity and may have an opposing surface adhering to an overlying non-adhesive inert layer. Optionally, such a transmucosal delivery system can be in the form of a bilayer tablet, in which the inner layer also contains additional binding agents, flavoring agents, or fillers. Some useful systems employ a non-ionic detergent along with a permeation enhancer. These examples are merely illustrative of available transmucosal delivery

technology and are not limiting of the present invention.

Another preferred embodiment of the composition of the present invention is a gel for systemic delivery of a phytosterol compound and a phytoestrogen compound via the rectal or vaginal mucosa, similar to gels commonly used for the delivery of various other therapeutic agents. Hydrogel matrices are known for this purpose. (Feijen, *Biodegradable hydrogel matrices for the controlled release of pharmacologically active agents*, U.S. Pat. No. 4,925,677). Such biodegradable gel matrices can be formed, for example, by cross-linking a proteinaceous component and a polysaccharide or mucopolysaccharide component, then loading with a phytosterol compound and a phytoestrogen compound to be delivered.

Another preferred embodiment of the composition of the present invention is the systemic delivery of a phytosterol compound and a phytoestrogen compound via a biodegradable matrix implanted within the body or under the skin of a human or non-human vertebrate. The implant matrix may be a hydrogel similar to those described above. Alternatively, it may be formed from a poly-alpha-amino acid component. (Sidman, *Biodegradable, implantable drug delivery device, and process for preparing and using same*, U.S. Pat. No. 4,351,337).

Another embodiment of the composition of the present invention employing a systemic delivery route is a transdermal delivery system of a kind known in the art for delivering various drugs. Transdermal delivery systems can be of any number of varieties known in the art. For example, delivery of a phytosterol compound and a phytoestrogen compound can be via a transdermal delivery system comprising a laminated composite of an adhesive layer, a backing layer, a permeable membrane defining a reservoir containing a phytosterol compound and a phytoestrogen compound, a peel seal disc underlying the membrane, one or more heat seals, and a removable release liner. (Ebert *et al.*, *Transdermal delivery system with adhesive overlay and peel seal disc*, U.S. Pat No. 5,662,925; Chang *et al.*, *Device for administering an active agent to the skin or mucosa*, U.S. Pat. Nos. 4,849,224 and 4,983,395).

Alternatively, a transdermal delivery device can be a matrix type transdermal patch. (Chien *et al.*, *Transdermal estrogen/progestin dosage unit, system and process*, U.S. Pat. Nos. 4,906,169 and 5,023,084; Cleary *et al.*, *Diffusion matrix for transdermal drug administration and transdermal drug delivery devices including same*, U.S. Pat. No. 4,911,916; Teillaud *et al.*, *EVA-based transdermal matrix system for the administration of an estrogen and/or a progestogen*, U.S. Pat. No. 5.605,702; Venkateshwaran *et al.*, *Transdermal drug delivery matrix for coadministering estradiol and another steroid*, U.S. Pat. No. 5,783,208; Ebert *et al.*, *Methods for providing testosterone and optionally estrogen replacement therapy to women*, U.S. Pat. No. 5,460,820). The matrix of the patch can comprise a basal support layer, such as an acrylic or ethylene/vinyl acetate copolymer or a polyurethane foam or cellulosic material, and an adhesive, such as, but not limited to, polysiloxane. In the compositions of the present invention, the polymer matrix also contains a phytosterol

compound and a phytoestrogen compound, as described above, and optionally, a penetration-enhancing vehicle or carrier, such as N-vinyl-2-pyrrolidone, N-(2-hydroxyethyl)-pyrrolidone, or 3-hydroxy-N-methyl-2-pyrrolidone. The adhesive patch may be pressure-sensitive, to release the phytosterol compound and a phytoestrogen compound across the skin of the patient when the patch matrix has been applied to the skin. The patch may optionally comprise an inert backing layer in addition to a matrix layer, or can comprise multiple dosage units or layers.

By using the present pharmaceutical compositions in accordance with the present method of inhibiting biosynthesis or bioactivity of an endogenous steroid sex hormone in a human subject, the process of steroidogenic biosynthesis of sex steroids is inhibited and/or the bioactivity of one or more endogenous steroid sex hormone(s) is inhibited (or reduced). Thus, the present invention provides the benefits of lowered risk with respect to cancers of the breast, endometrium, and prostate, and of BPH, while simultaneously preserving the benefits to cardiovascular and skeletal health, libido and energy, and cognitive function that are associated with aromatizable androgens and estrogens.

The foregoing being illustrative but not an exhaustive description of the embodiments of the present invention, the following claims are presented.

CLAIMS

1. A method of inhibiting biosynthesis or bioactivity of an endogenous sex steroid hormone in a human subject, comprising:

administering to a human subject a combination of a dose of at least one phytosterol compound together with a dose of at least one phytoestrogen compound in an amount sufficient to inhibit biosynthesis or bioactivity of an endogenous steroid sex hormone.

2. The method of Claim 1, wherein said phytosterol compound is campesterol, sitosterol, fucosterol, stigmasterol, stigmastanol, or stigmastadienone, or a derivative or conjugate of any of these.

3. The method of Claim 1, wherein said phytosterol compound is β-sitosterol or a derivative or conjugate thereof.

4. The method of Claim 1, wherein the phytoestrogen compound is a coumestan, lignan, flavone, or isoflavone compound, or a derivative or conjugate of any of these.

5. The method of Claim 1, wherein the phytoestrogen compound is sesamin, justiciresinol, lariciresinol, isolariciresinol, secoisolariciresinol, O-demethylsecoisolariciresinol, didemethylsecoisolariciresinol, demethoxysecoisolariciresinol, matairesinol, syringaresinol, episyringaresinol, diasyringaresinol, massoniresinol, lirioresinol, entrodiol, enterolactone, gomisin A, gomisin C, gomisin D, nordihydroguaiaretic acid, 3'-O-methyl nordihydroguaiaretic acid, arctigenin, or 3'-O-demethylarctigenin, or a derivative or conjugate of any of these.

6. The method of Claim 1, wherein the phytoestrogen compound is syringaresinol-β-D-glucoside, massoniresinol-4"-O-β-D-glucopyranoside, secoisolariciresinol diglycoside, or ramontoside.

7. The method of Claim 1, wherein the phytoestrogen compound is genistein, daidzein, biochanin A, glycitein, zearalenone, beta-zearalenol, formononetin, O-desmethylangolensin, ipriflavone, apigenin, phloretin, baicalein, alpinumisoflavone, hydroxyalpinumisoflavone, or a derivative or conjugate of any of these.

8. The method of Claim 1, wherein the phytoestrogen compound is dihydrodaidzein, tetrahydrodaidzein, dihydrogenistein, or 2-dehydro-O-demethylangolensin.

9. The method of Claim 1, wherein the phytoestrogen compound is kaempferol, galangin, fisetin, morin, chrysin, tectochrysin, isoprunetin, wighteone, laburnetin, diosmetin, genistin, genisteone, ephedroidin, baicalin, puerarin, poncirin, hesperidin, naringin, isorhamnetin, norwogenin, 2,5-dihydroxy-6,7-dimethoxyflavonone, xanthohumol, isoxanthohumol, desmethoxyxanthohumol, naringenin, or 6-geranylnaringenin, or a derivative or conjugate of any of these.

10. The method of Claim 1, wherein the phytoestrogen compound is coumestrol, quercetin, resveratrol, rutin, myricetin, or luteolin, or a derivative or conjugate of any of these.

11. The method of Claim 1, wherein the phytoestrogen compound is 3-O-methylquercetin, 3',4'-di-O-benzyl-3-O-methylquercetin, quercetin-3-O-β-D-glucopyranoside, quercetin-4'-O-β-D-glucopyranoside, quercetin-3,4-diO-β-D-glucoside, quercetin-7-O-β-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranoside, isorhamnetin-4'-O-β-glucoside, or 4-(G)-α-glucopyranosylrutin.

12. The method of Claim 1, wherein the phytosterol compound is administered in a dose of 1 to 5 milligrams per kilogram of body weight per day, and the phytoestrogen compound is administered in a dose of 0.5 to 2 milligrams per kilogram of body weight per day.

13. The method of Claim 1, wherein the dose of the phytosterol compound(s) and the dose of the phytoestrogen compound(s) are delivered substantially simultaneously by one or more systemic delivery route(s).

14. The method of Claim 13, wherein the systemic delivery route is by ingestion, injection, intravenous drip, or implant.

15. The method of Claim 13, wherein the systemic delivery route is a transdermal delivery route.

16. The method of Claim 13, wherein the systemic delivery route is a transmucosal delivery route.

17. A method of inhibiting biosynthesis or bioactivity of an endogenous steroid sex hormone in a human subject, comprising:

administering to a human subject a pharmaceutically acceptable composition comprising a combination of a dose of at least one phytosterol compound and a dose of at least one phytoestrogen compound in an amount sufficient to inhibit biosynthesis or bioactivity of an endogenous steroid sex hormone.

18. The method of Claim 17, wherein said phytosterol compound is campesterol, sitosterol, fucosterol, stigmasterol, stigmastanol, or stigmastadienone, or a derivative or conjugate of any of these.

19. The method of Claim 17, wherein said phytosterol compound is β-sitosterol or a derivative or conjugate thereof.

20. The method of Claim 17, wherein the phytoestrogen compound is a coumestan, lignan, flavone, or isoflavone compound, or a derivative or conjugate of any of these.

21. The method of Claim 17, wherein the phytoestrogen compound is sesamin, justiciresinol, lariciresinol, isolariciresinol, secoisolariciresinol, O-demethylsecoisolariciresinol, didemethylsecoisolariciresinol, demethoxysecoisolariciresinol, matairesinol, syringaresinol, episyringaresinol, diasyringaresinol, massoniresinol, lirioresinol, entrodiol, enterolactone, gomisin A, gomisin C, gomisin D, nordihydroguaiaretic acid, 3'-O-methyl nordihydroguaiaretic acid, arctigenin, or 3'-O-demethylarctigenin, or a derivative or conjugate of any of these.

22. The method of Claim 17, wherein the phytoestrogen compound is syringaresinol-β-D-glucoside, massoniresinol-4"-O-β-D-glucopyranoside, secoisolariciresinol diglycoside, or ramontoside.

23. The method of Claim 17, wherein the phytoestrogen compound is genistein, daidzein, biochanin A, glycitein, zearalenone, beta-zearalenol, formononetin, O-desmethylangolensin, ipriflavone, apigenin, phloretin, baicalein, alpinumisoflavone, hydroxyalpinumisoflavone, or a derivative or conjugate of any of these.

24. The method of Claim 17, wherein the phytoestrogen compound is dihydrodaidzein, tetrahydrodaidzein, dihydrogenistein, or 2-dehydro-O-demethylangolensin.

25. The method of Claim 17, wherein the phytoestrogen compound is kaempferol, galangin, fisetin, morin, chrysin, tectochrysin, isoprunetin, wighteone, laburnetin, diosmetin, genistin, genisteone, ephedroidin, baicalin, puerarin, poncirin, hesperidin, naringin, isorhamnetin, norwogenin, 2,5-dihydroxy-6,7-dimethoxyflavonone, xanthohumol, isoxanthohumol, desmethoxyxanthohumol, naringenin, or 6-geranylnaringenin, or a derivative or conjugate of any of these.

26. The method of Claim 17, wherein the phytoestrogen compound is coumestrol, quercetin, resveratrol, rutin, myricetin, or luteolin, or a derivative or conjugate of any of these.

27. The method of Claim 17, wherein the phytoestrogen compound is 3-O-methylquercetin, 3',4'-di-O-benzyl-3-O-methylquercetin, quercetin-3-O-β-D-glucopyranoside, quercetin-4'-O-β-D-glucopyranoside, quercetin-3,4-diO-β-D-glucoside, quercetin-7-O-β-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranoside, isorhamnetin-4'-O-β-glucoside, or 4-(G)-α-glucopyranosylrutin.

28. The method of Claim 17, wherein the phytosterol compound is administered in a dose of 1 to 5 milligrams per kilogram of body weight per day, and the phytoestrogen compound is administered in a dose of 0.5 to 2 milligrams per kilogram of body weight per day.

29. The method of Claim 17, wherein said composition is administered by a systemic delivery route.

30. The method of Claim 29, wherein said systemic delivery route is by ingestion,

injection, intravenous drip, or implant.

31. The method of Claim 29, wherein said systemic delivery route is a transdermal delivery route.

32. The method of Claim 29, wherein said systemic delivery route is a transmucosal delivery route.

33. The method of Claim 17, wherein said composition is administered as a food additive or food supplement.

34. A method of inhibiting biosynthesis or bioactivity of an endogenous steroid sex hormone in a human subject, comprising:

administering to a human subject a pharmaceutically acceptable composition comprising a combination of at least one phytosterol compound and at least one phytoestrogen compound, such that the phytosterol compound is delivered in a dose of 1 to 5 milligrams per kilogram of body weight per day, and the phytoestrogen compound is delivered in a dose of 0.5 to 2 milligrams per kilogram of body weight per day, whereby biosynthesis or bioactivity of an endogenous steroid sex hormone is inhibited in said human.

35. The method of Claim 34, wherein said phytosterol compound is campesterol, sitosterol, fucosterol, stigmasterol, stigmastanol, or stigmastadienone, or a derivative or conjugate of any of these.

36. The method of Claim 34, wherein said phytosterol compound is β-sitosterol or a derivative or conjugate thereof.

37. The method of Claim 34, wherein the phytoestrogen compound is a coumestan, lignan, flavone, or isoflavone compound, or a derivative or conjugate of any of these.

38. The method of Claim 34, wherein the phytoestrogen compound is sesamin.

justiciresinol, lariciresinol, isolariciresinol, secoisolariciresinol, O-demethylsecoisolariciresinol, didemethylsecoisolariciresinol, demethoxysecoisolariciresinol, matairesinol, syringaresinol, episyringaresinol, diasyringaresinol, massoniresinol, lirioresinol, entrodiol, enterolactone, gomisin A, gomisin C, gomisin D, nordihydroguaiaretic acid, 3'-O-methyl nordihydroguaiaretic acid, arctigenin, or 3'-O-demethylarctigenin, or a derivative or conjugate of any of these.

39. The method of Claim 34, wherein the phytoestrogen compound is syringaresinol-β-D-glucoside, massoniresinol-4"-O-β-D-glucopyranoside, secoisolariciresinol diglycoside, or ramontoside.

40. The method of Claim 34, wherein the phytoestrogen compound is genistein, daidzein, biochanin A, glycitein, zearalenone, beta-zearalenol, formononetin, O-desmethylangolensin, ipriflavone, apigenin, phloretin, baicalein, alpinumisoflavone, hydroxyalpinumisoflavone, or a derivative or conjugate of any of these.

41. The method of Claim 34, wherein the phytoestrogen compound is dihydrodaidzein, tetrahydrodaidzein, dihydrogenistein, or 2-dehydro-O-demethylangolensin.

42. The method of Claim 34, wherein the phytoestrogen compound is kaempferol, galangin, fisetin, morin, chrysin, tectochrysin, isoprunetin, wighteone, laburnetin, diosmetin, genistin, genisteone, ephedroidin, baicalin, puerarin, poncirin, hesperidin, naringin, isorhamnetin, norwogenin, 2,5-dihydroxy-6,7-dimethoxyflavonone, xanthohumol, isoxanthohumol, desmethoxyxanthohumol, naringenin, or 6-geranylnaringenin, or a derivative or conjugate of any of these.

43. The method of Claim 34, wherein the phytoestrogen compound is coumestrol, quercetin, resveratrol, rutin, myricetin, or luteolin, or a derivative or conjugate of any of these.

44. The method of Claim 34, wherein the phytoestrogen compound is 3-O-methylquercetin 3',4'-di-O-benzyl-3-O-methylquercetin, quercetin-3-O-β-D-glucopyranoside, quercetin-4'-O-β-D-glucopyranoside, quercetin-3,4-diO-β-D-glucoside, quercetin-7-O-β-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranoside, isorhamnetin-4'-O-β-glucoside, or 4-(G)-α-glucopyranosylrutin.

45. The method of Claim 34, wherein said composition is administered by a systemic delivery route.

46. The method of Claim 45, wherein said systemic delivery route is by ingestion, injection, intravenous drip, or implant.

47. The method of Claim 45, wherein said systemic delivery route is a transdermal delivery route.

48 The method of Claim 45, wherein said systemic delivery route is a transmucosal delivery route.

49. The method of Claim 34, wherein said composition is administered as a food supplement or food additive.

50. A pharmaceutical composition comprising a combination of at least one phytosterol compound and at least one phytoestrogen compound formulated in a delivery system to deliver a dose of 50 to 250 mg of phytosterol compound(s) and 20 to 150 mg of phytoestrogen compound(s).

51. The pharmaceutical composition of Claim 50, formulated in a delivery system to deliver a dose of 175 to 225 milligrams of the phytosterol compound(s) and 75 to 125 milligrams of the phytoestrogen compound(s).

52. The pharmaceutical composition of Claim 50, wherein said phytosterol compound is campesterol, sitosterol, fucosterol, stigmasterol, stigmastanol, or stigmastadienone, or a derivative or conjugate of any of these.

53. The pharmaceutical composition of Claim 50, wherein said phytosterol compound is β-sitosterol or a derivative or conjugate thereof.

54. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound

is a lignan, isoflavone, flavone, or coumestan compound, or a derivative or conjugate of any of these.

55. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is sesamin, justiciresinol, lariciresinol, isolariciresinol, secoisolariciresinol, O-demethylsecoisolariciresinol, didemethylsecoisolariciresinol, demethoxysecoisolariciresinol, matairesinol, syringaresinol, episyringaresinol, diasyringaresinol, massoniresinol, lirioresinol, entrodiol, enterolactone, gomisin A, gomisin C, gomisin D, nordihydroguaiaretic acid, 3'-O-methyl nordihydroguaiaretic acid, arctigenin, or 3'-O-demethylarctigenin, or a derivative or conjugate of any of these.

56. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is syringaresinol-β-D-glucoside, massoniresinol-4"-O-β-D-glucopyranoside, secoisolariciresinol diglycoside, or ramontoside.

57. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is genistein, daidzein, biochanin A, glycitein, zearalenone, beta-zearalenol, formononetin, O-desmethylangolensin, ipriflavone, apigenin, phloretin, baicalein, alpinumisoflavone, hydroxyalpinumisoflavone, or a derivative or conjugate of any of these.

58. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is dihydrodaidzein, tetrahydrodaidzein, dihydrogenistein, or 2-dehydro-O-demethylangolensin.

59. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is kaempferol, galangin, fisetin, morin, chrysin, tectochrysin, isoprunetin, wighteone, laburnetin, diosmetin, genistin, genisteone, ephedroidin, baicalin, puerarin, poncirin, hesperidin, naringin, isorhamnetin, norwogenin, 2,5-dihydroxy-6,7-dimethoxyflavonone, xanthohumol, isoxanthohumol, desmethoxyxanthohumol, naringenin, or 6-geranylnaringenin, or a derivative or conjugate of any of these.

60. The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is coumestrol, quercetin, resveratrol, rutin, myricetin, or luteolin, or a derivative or conjugate of any of these.

61 The pharmaceutical composition of Claim 50, wherein the phytoestrogen compound is 3-O-methylquercetin, 3',4'-di-O-benzyl-3-O-methylquercetin, quercetin-3-O-β-D-glucopyranoside, quercetin-4'-O-β-D-glucopyranoside, quercetin-3,4-diO-β-D-glucoside, quercetin-7-O-β-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranoside, isorhamnetin-4'-O-β-glucoside, or 4-(G)-α-glucopyranosylrutin.

62. The pharmaceutical composition of Claim 50, formulated for a systemic delivery system.

63. The pharmaceutical composition of Claim 50, formulated for an ingestive, injection, intravenous drip, or implant delivery system.

64. The pharmaceutical composition of Claim 50, formulated for a transdermal delivery system.

65. The pharmaceutical composition of Claim 50, formulated for a transmucosal delivery system.

66. The pharmaceutical composition of Claim 50, formulated for delivery as a powder, tablet, capsule, or caplet.